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RESEARCH ON DETECTION OF EXTRATERRESTRIAL LIFE BY ULTRAVIOLET SPECTROPHOTOMETRY

SECOND QUARTERLY PROGRESS REPORT

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INTRODUCTION

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The work described in this report is part of a program on the study of absorption of a narrow region of the far ultraviolet by materials of biological origin. It is based on reported observations[1,2,3] that the peptide bond exhibits a characteristic absorption of ultraviolet light of 185 to 190 millimicrons.

The measurements reported in the first quarterly report were obtained on a Cary model 15 spectrophotometer. Absorption maxima between 185 and 190 mμ were found for phenyl alanine, tryptophan, tyrosine, serum albumin and ribonuclease. In the case of the two proteins, the absorbancies were studied as a function of pH and were found to pass through a maximum at approximately pH 7. Absorption maxima at the wavelength of interest were not observed with glycine, alanine, or glycyl glycine.

The work described in the present report is a continuation of the author previous work.

EXPERIMENTAL

Instrumental

All absorption measurements were made with a Beckman DK-2A, modified for far ultraviolet under conditions of continuous flushing with nitrogen. Fused silica cells (transmitting ultraviolet down to 180 mμ) were used with inserts to achieve a path length of .05 cm.

Materials

The following materials were studied spectrophotometrically

DL-alanyl-DL-alanine

DL-alanyl-glycine

DL-alanyl-DL-leucine

DL-leucyl-glycyl-glycine

DL-alanyl-glycyl-glycine

Poly-L-lysine

Poly-L-glutamic acid

Polyglycine

Bovine serum albumin

Extracts of local soil

Methods

Solutions were made in phosphate buffers of various pH values, sodium hydroxide, sulfuric acid, and hydrochloric acid. Phosphate buffer and hydrochloric acid were suitable solvents for the dipeptides, tripeptides, some polypeptides, and albumin. Concentrated ammonium hydroxide was used as a solvent for the polyglutamic acid; sulfuric acid was used for the polyglycine.

In the case of the local soil samples, three aliquots of 25 gms were extracted; one in distilled water, one in sodium hydroxide and one in hydrocholoric acid. The extractions were carried out by stirring with the solvent for 30 minutes followed by filtration. The filtration was carried out with a No. 42 filter and then with a millipore filter.

In all cases, absorption spectra were obtained at several concentrations prepared by dilution of the most concentrated solution.

RESULTS

Peptides

All absorption measurements made on the peptides showed one strong absorption in the region of 185-190 mμ.

DL-alanyl-DL-alanine shows a pronounced absorption maximum in the wavelength region being studied. The spectrum shown in figure 1 is at pH 1, but figure 2 shows that the absorption at 190 mμ at other pH values follows Beer's law with the possible exception of pH 11.0. The effect of pH on the absorptivity and absorption maxima is illustrated in figure 3, showing a gradual shift from a higher wavelength to a lower wavelength and then back to a higher wavelength again as pH increases.

The spectrophotometric absorption by tripeptides is illustrated by measurements on DL-alanyl-glycyl-glycine. The spectrum in figure 4 shows the characteristic absorption between 185 and 190 mμ. The Beer's law plot in figure 5 for several pH valves indicates a true absorption again with the possible exception of pH 11. It may be seen in figure 6 that the absorption maximum undergoes a shift from higher wavelengths to lower ones and then back to higher ones as the pH is increased, in the same manner as the dipeptides.

The polypeptides resemble the smaller molecules in many respects. As an illustration, poly-L-lysine shows a strong absorption maximum in the same region as do the smaller molecules, as will be seen in figure 7. The absorption follows that predicted by Beer's law within a somewhat restricted concentration range as seen in figure 8. A study of the absorption maximum as a function of pH again shows a shift from longer

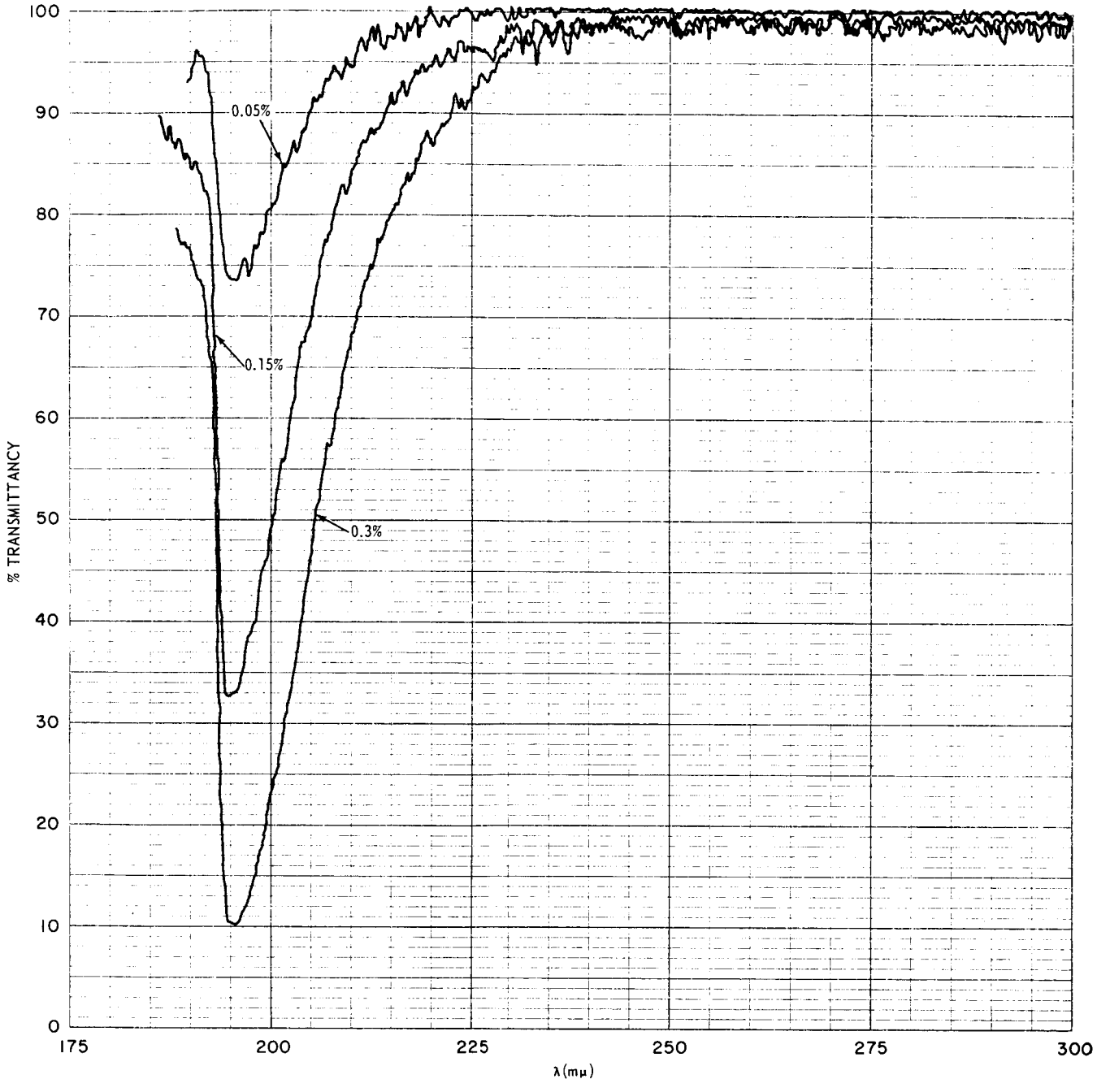

Figure 1. Absorption Spectra at pH 1 - Alanylalanine

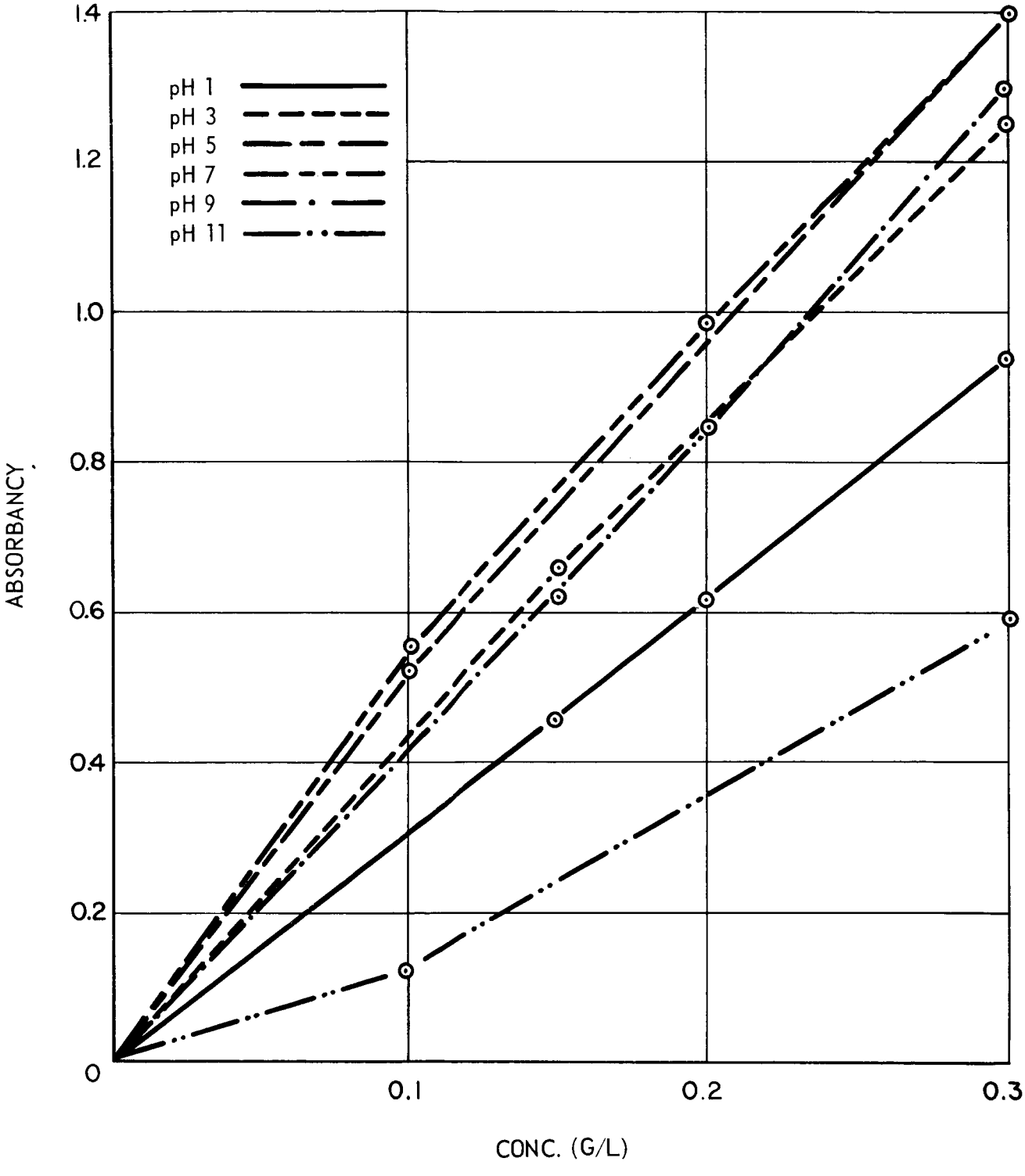

Figure 2. Beer's Law Plot of Alanylalanine at Different pH Factors

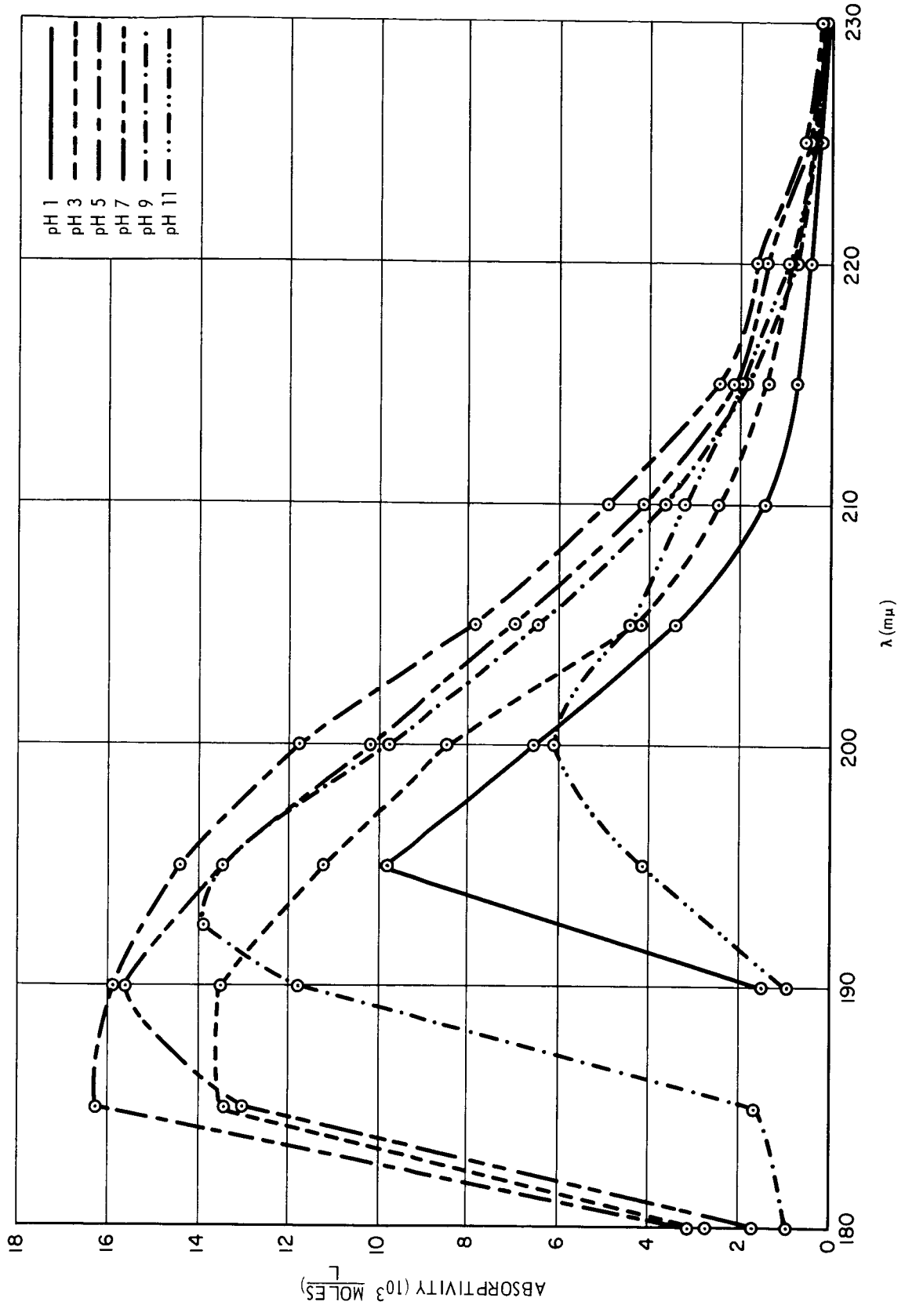

Figure 3. Effect of pH on Absorptivity by Alanylalanine

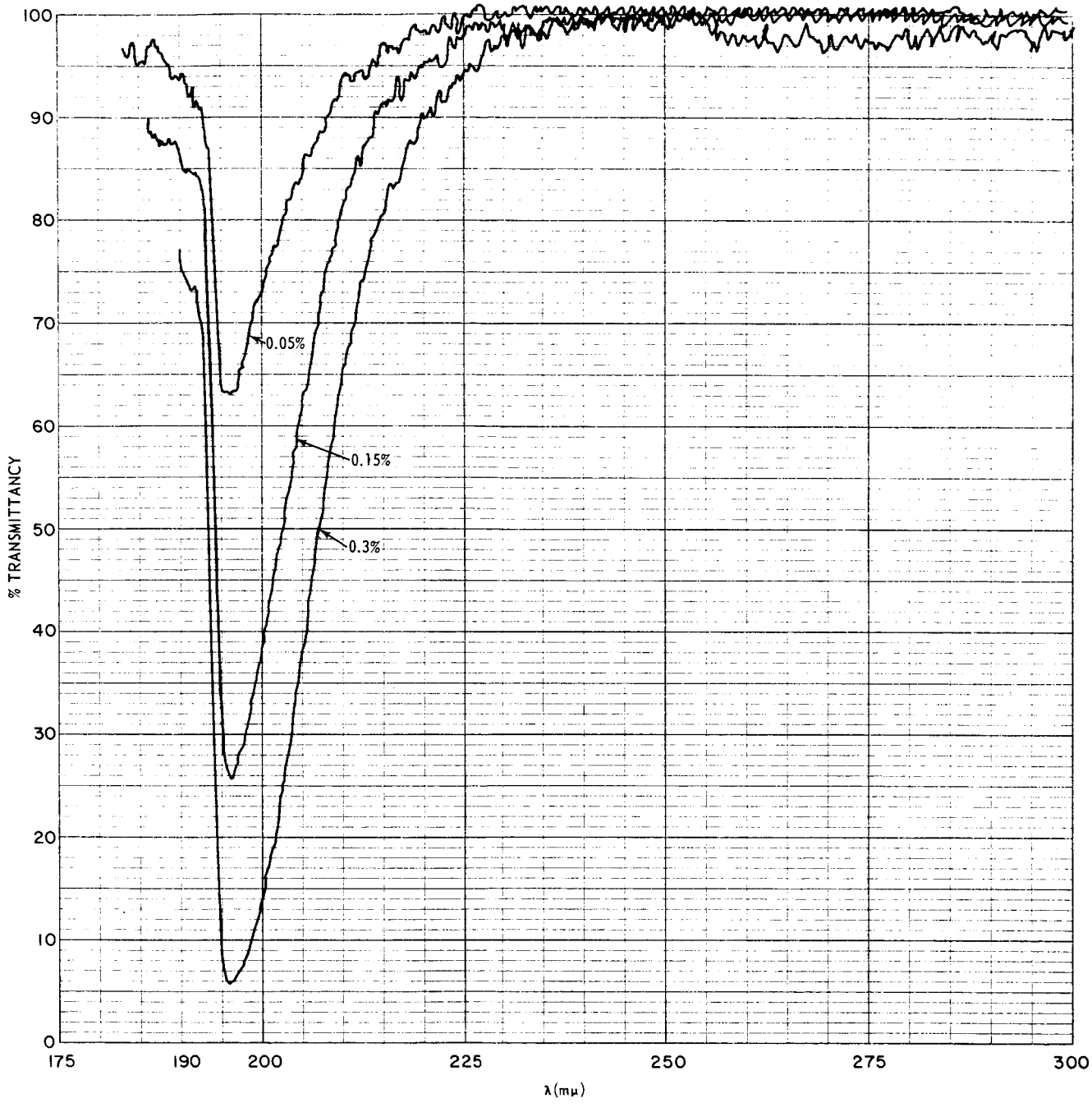

Figure 4. Absorption Spectra of pH 1 - Alanylglycylglycine

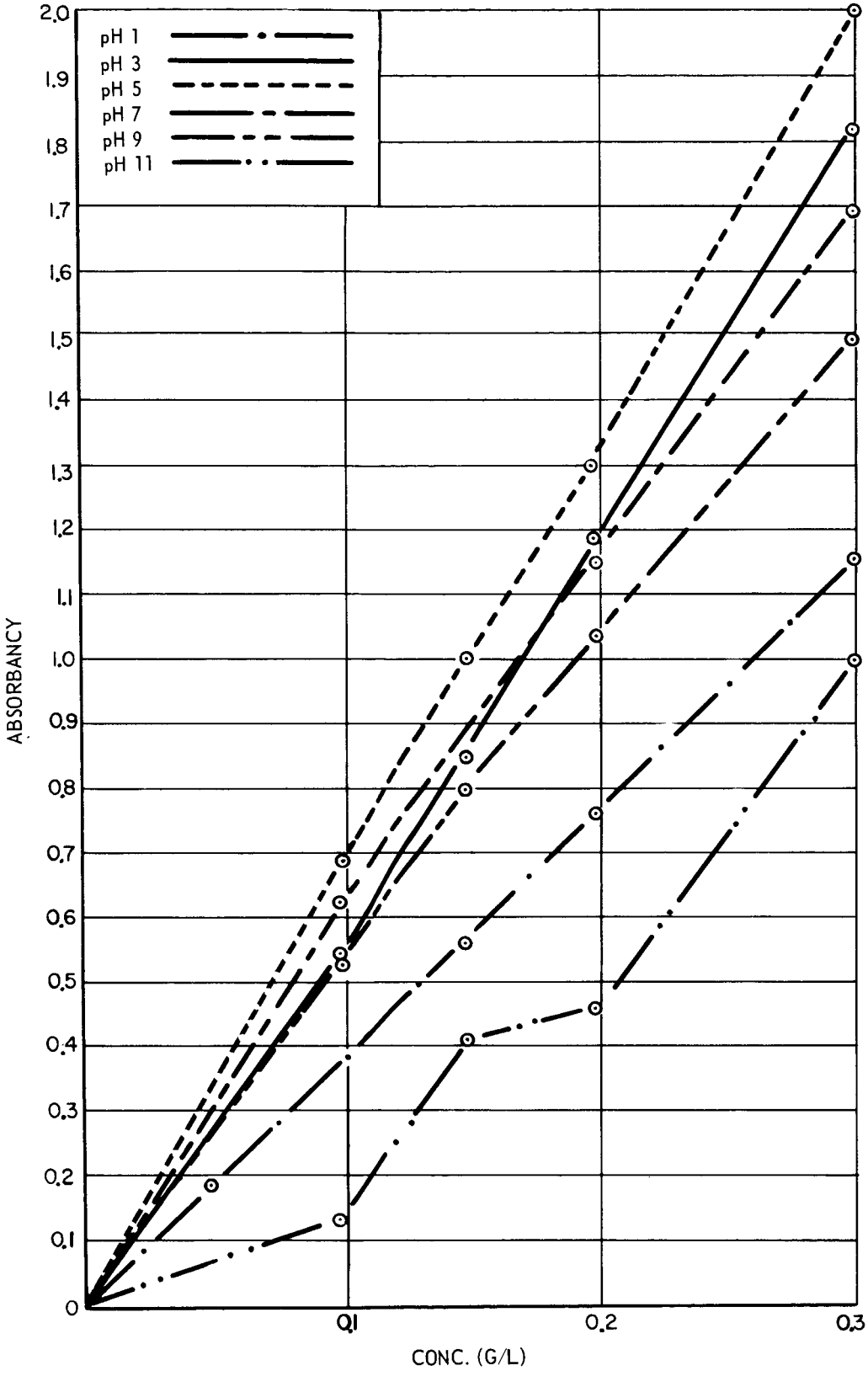

Figure 5. Beer's Law Plot of Alanylglycylglycine at Different pH Factors

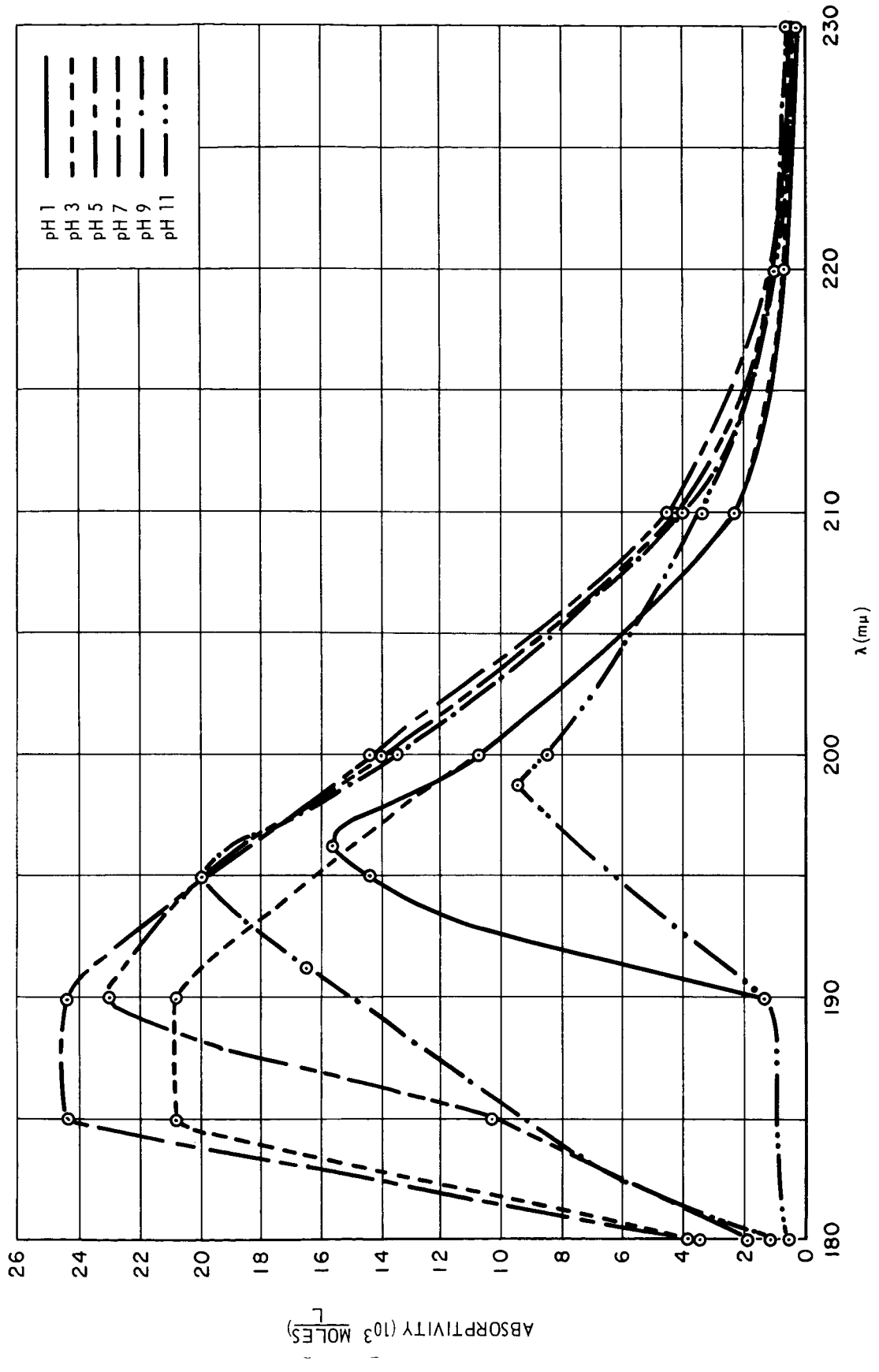

Figure 6. Effect of pH on Absorptivity by Alanylglycylglycine

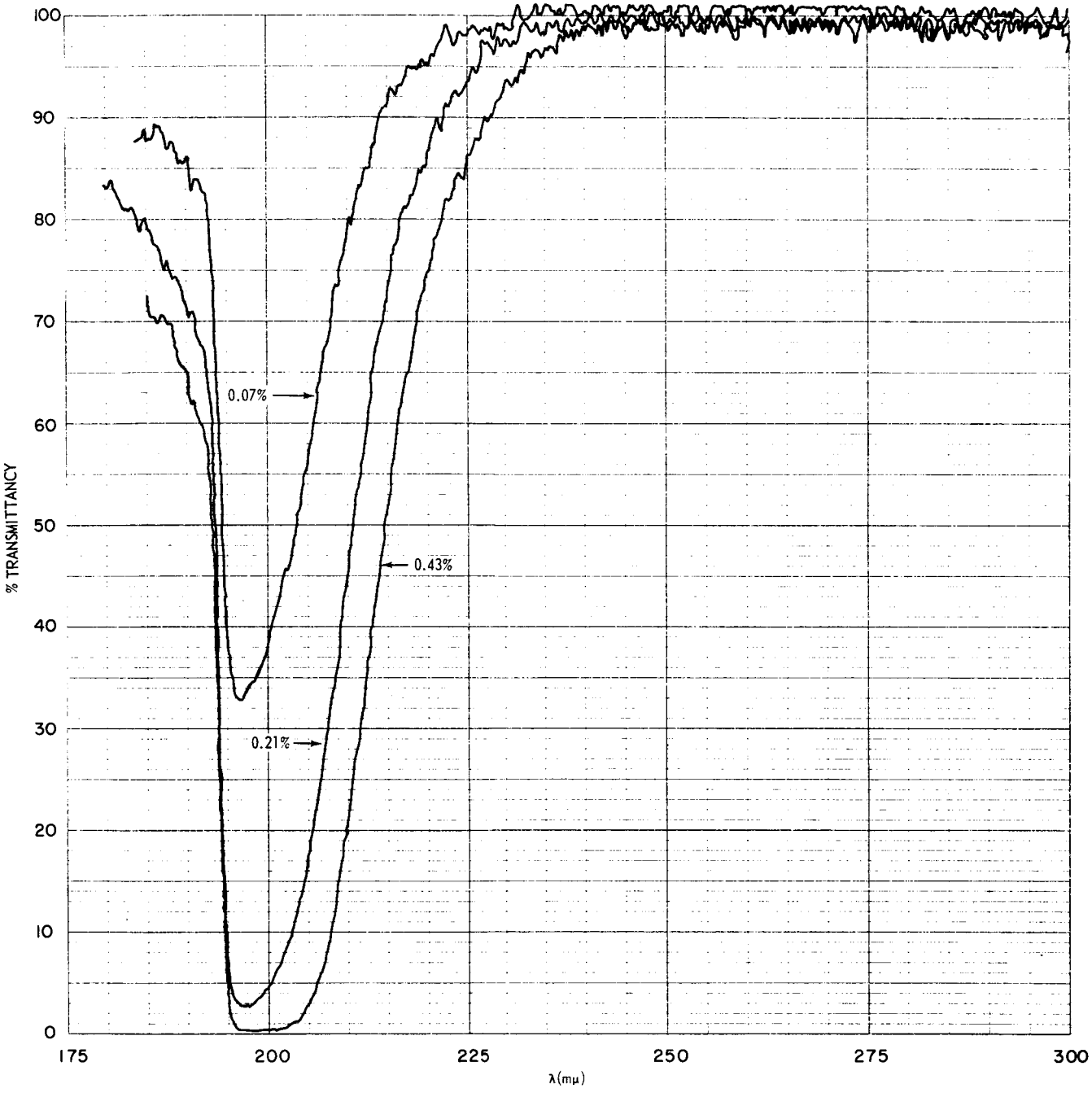

Figure 7. Absorption Spectra at pH 1 - Polylysine

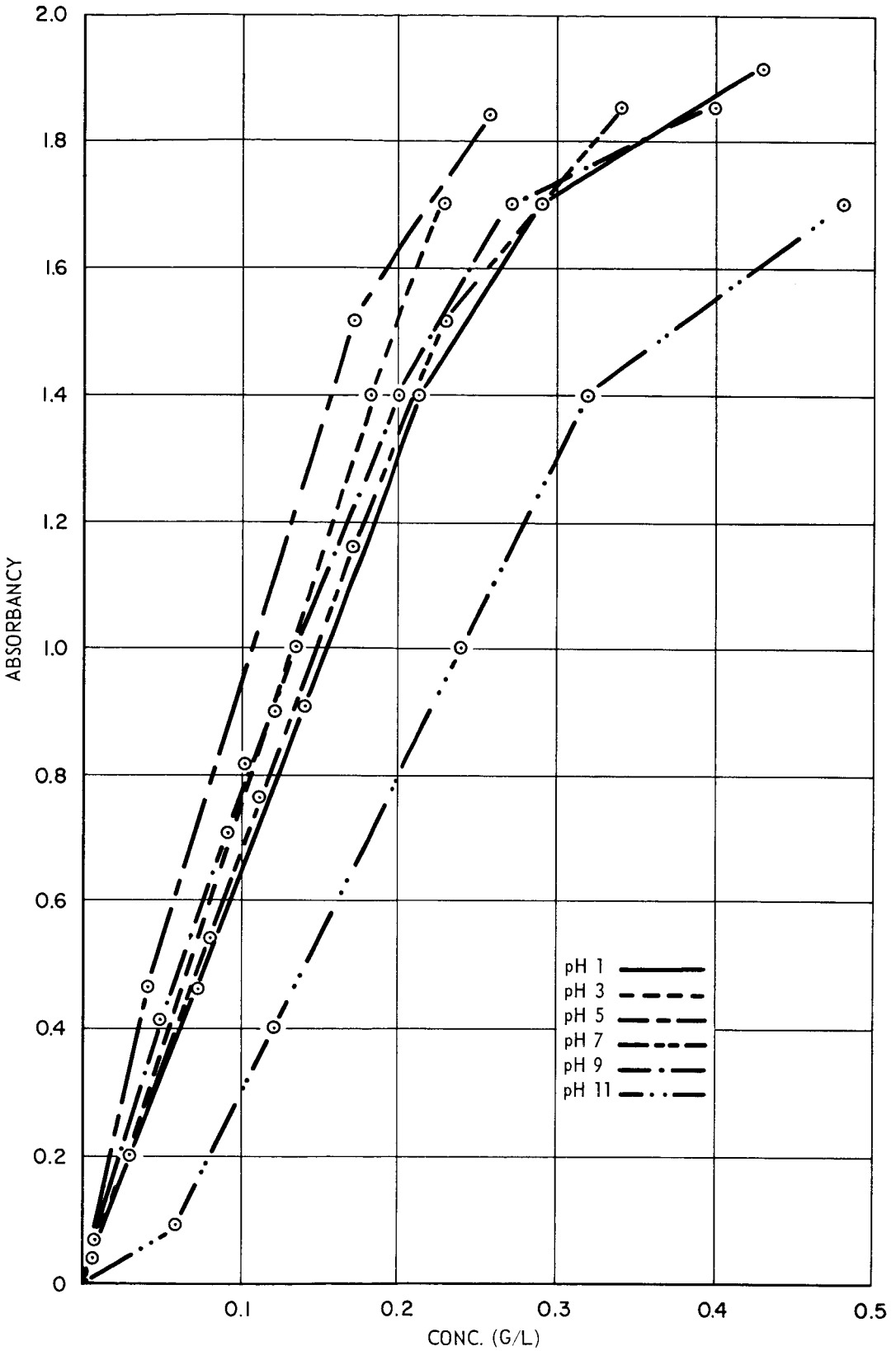

Figure 8. Beer's Law Plot of Polylysine at Different pH Factors

wavelengths to shorter ones and then back to longer wavelengths as the pH is increased (figure 9).

Protein

The absorption spectrum for bovine serum albumin shows two maxima, as seen in figure 10. One appears at about 280 mμ while the other is seen in the region of 185 to 190 mμ. The section in the insert is a ten-fold expansion of the absorption at 280 mμ, so that the absorption intensities of the two maxima may be compared. It is evident that the absorption at the shorter wavelength is much more intense than that at 280 mμ. The Beer's law plot for the protein at 190 mμ indicates a true absorption with some minor deviation at pH 11 (figure 11). Figure 12 again shows that the effect of pH on the absorptivity of the protein is the same as that observed before - namely, that there is a gradual shift from a higher wavelength to a lower one, and then back to a higher wavelength again.

Soil Extract

Significant absorption maxima in the wavelength region being studied were also obtained with the extracts from soil. Figure 13 shows these absorptions (at 180 mμ and 190 mμ) for the water and hydrochloric acid extracts, while figure 14 shows the absorption for the sodium hydroxide extracts, which seems to be at 198 mμ.

General

The effect of pH is generally similar for all the peptides and proteins studied to date. Figure 15 shows that in all cases the absorptivity reaches

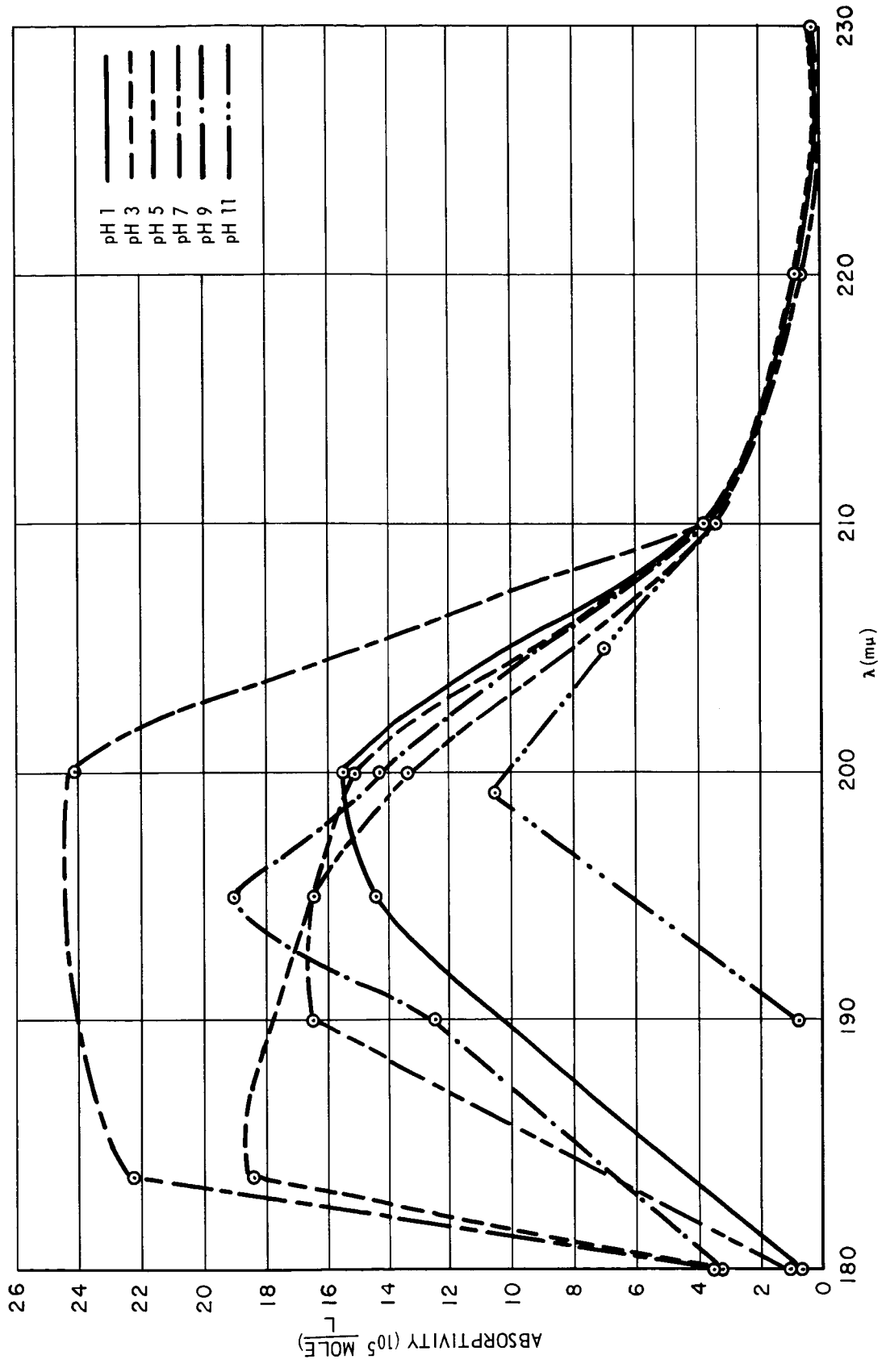

Figure 9. Effect of pH on Absorptivity by Polylysine

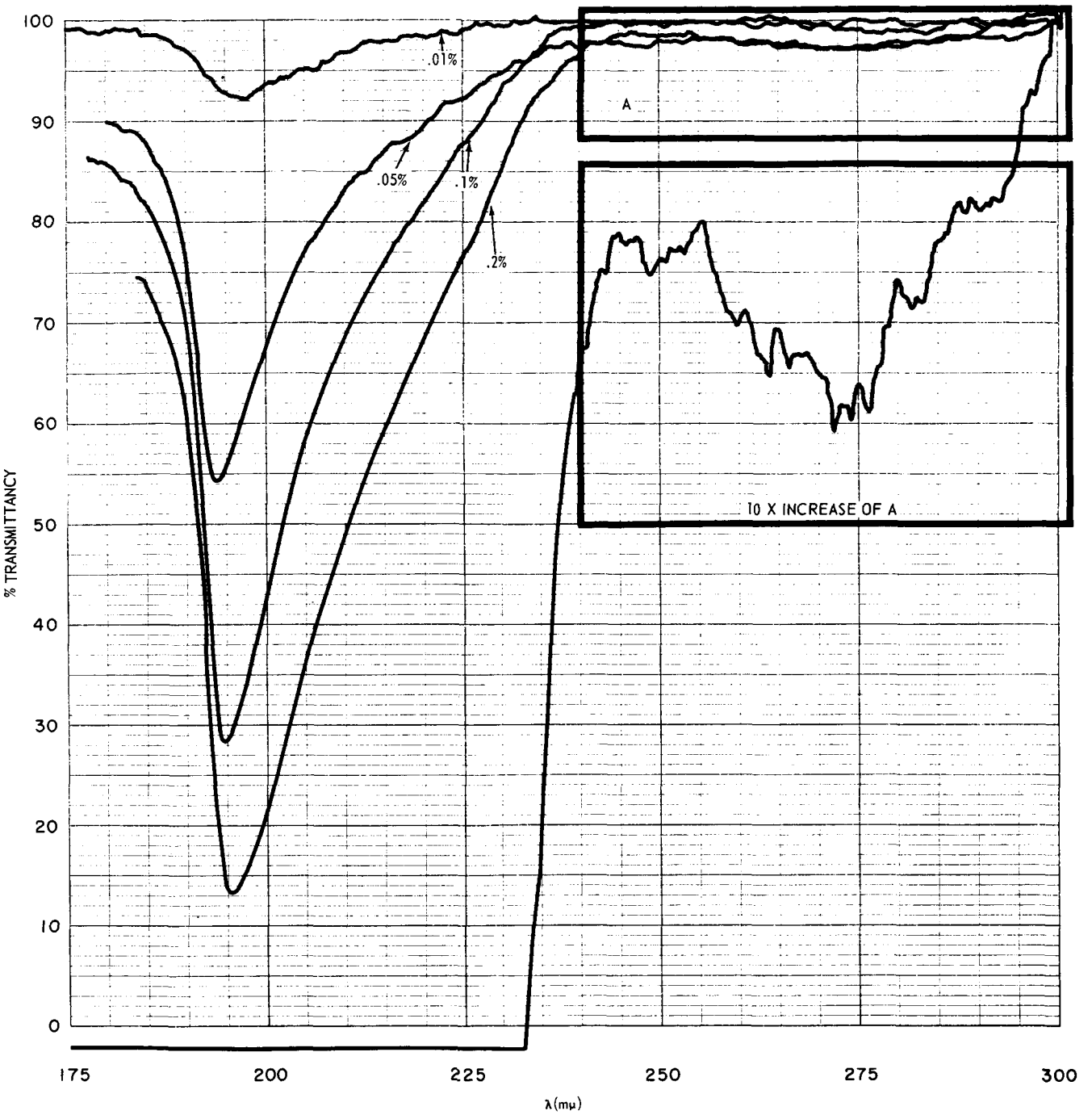

Figure 10. Absorption Spectra at pH 1 - Bovine Serum Albumin

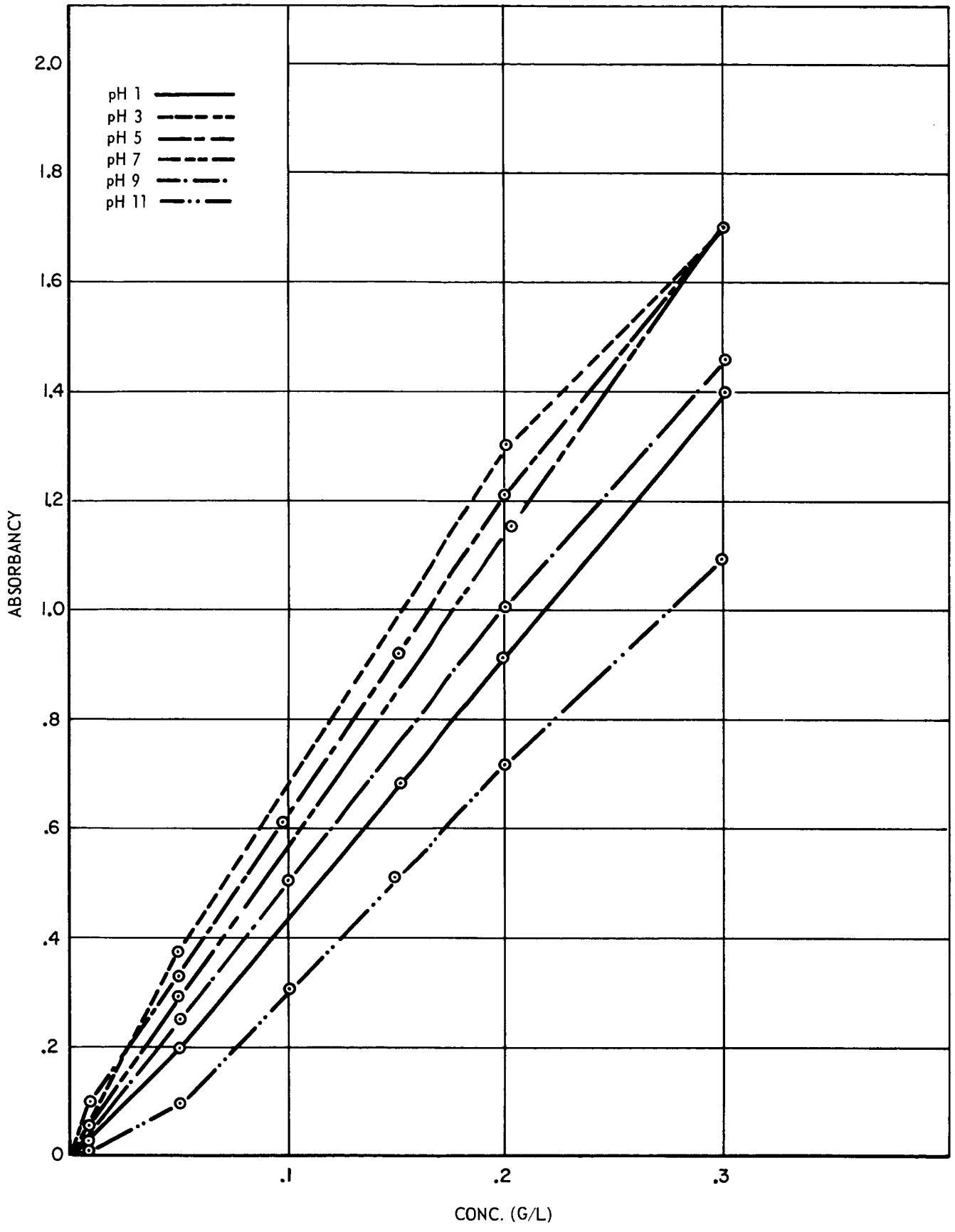

Figure 11. Beer's Law Plot of Bovine Serum Albumin of Different pH Factors

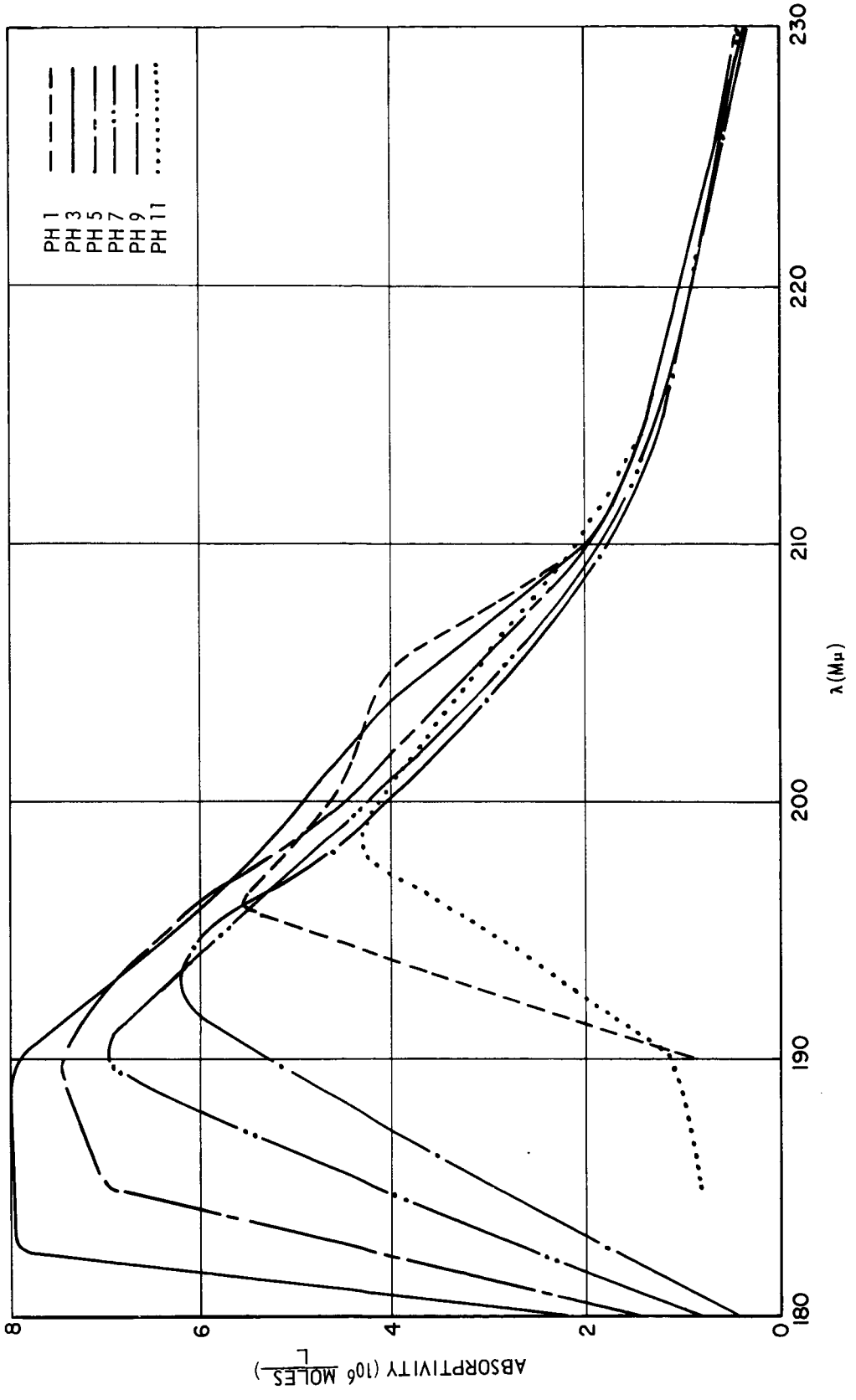

Figure 12. Effect of pH on Absorptivity by Bovine Serum Albumin

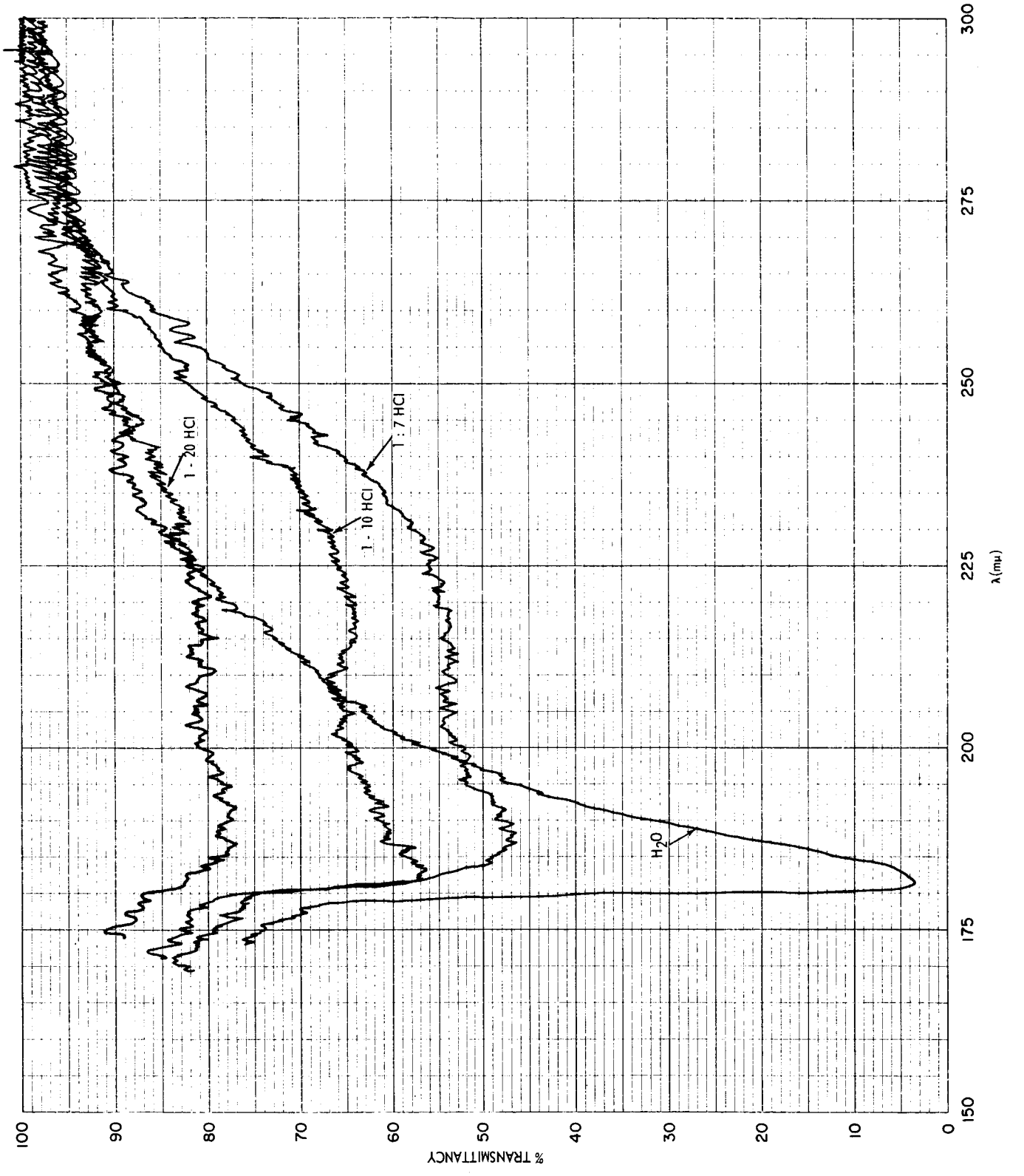

Figure 13. Absorption Spectra - Soil Extract

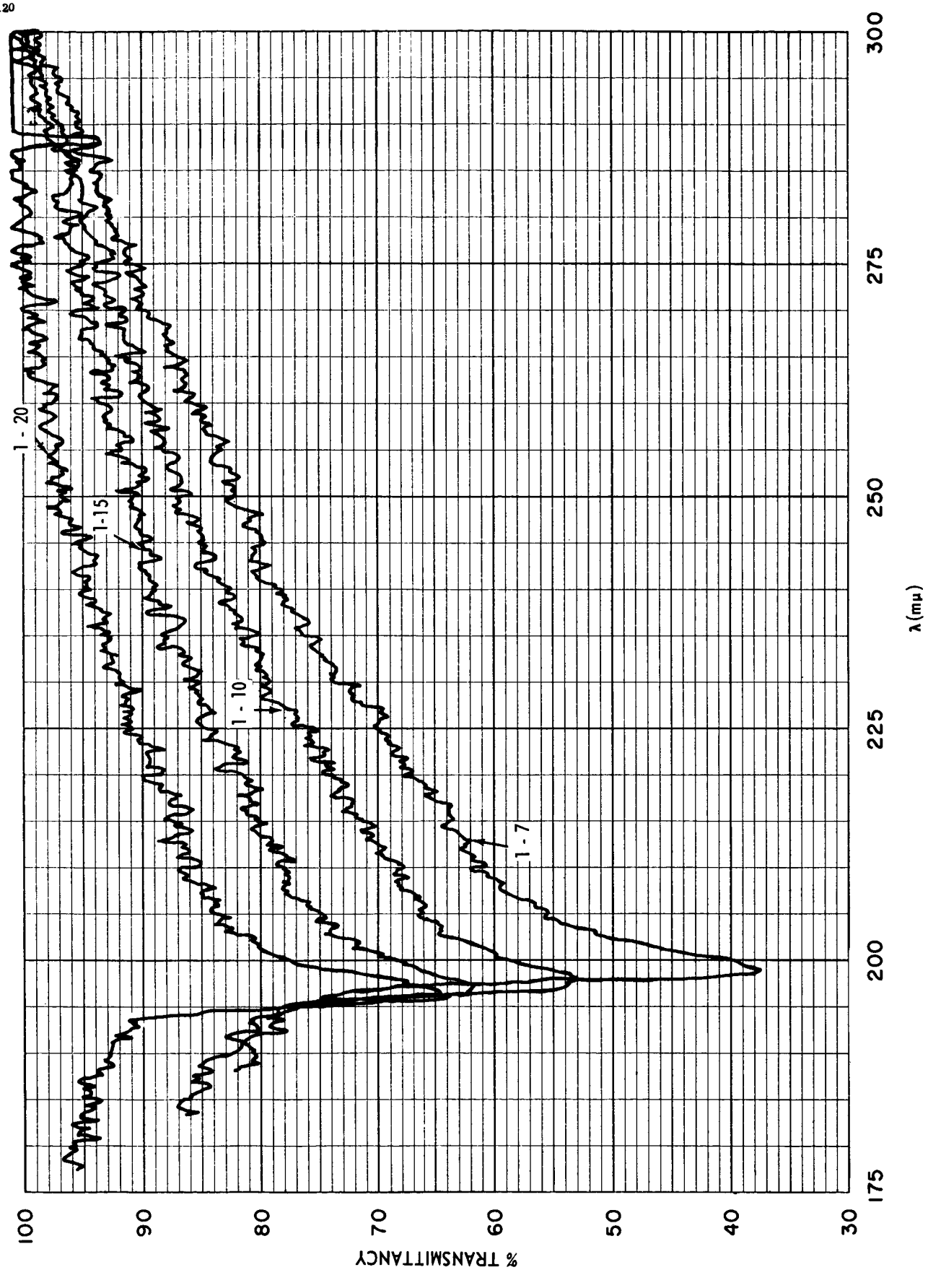

Figure 14. Absorption Spectra - Soil Extract

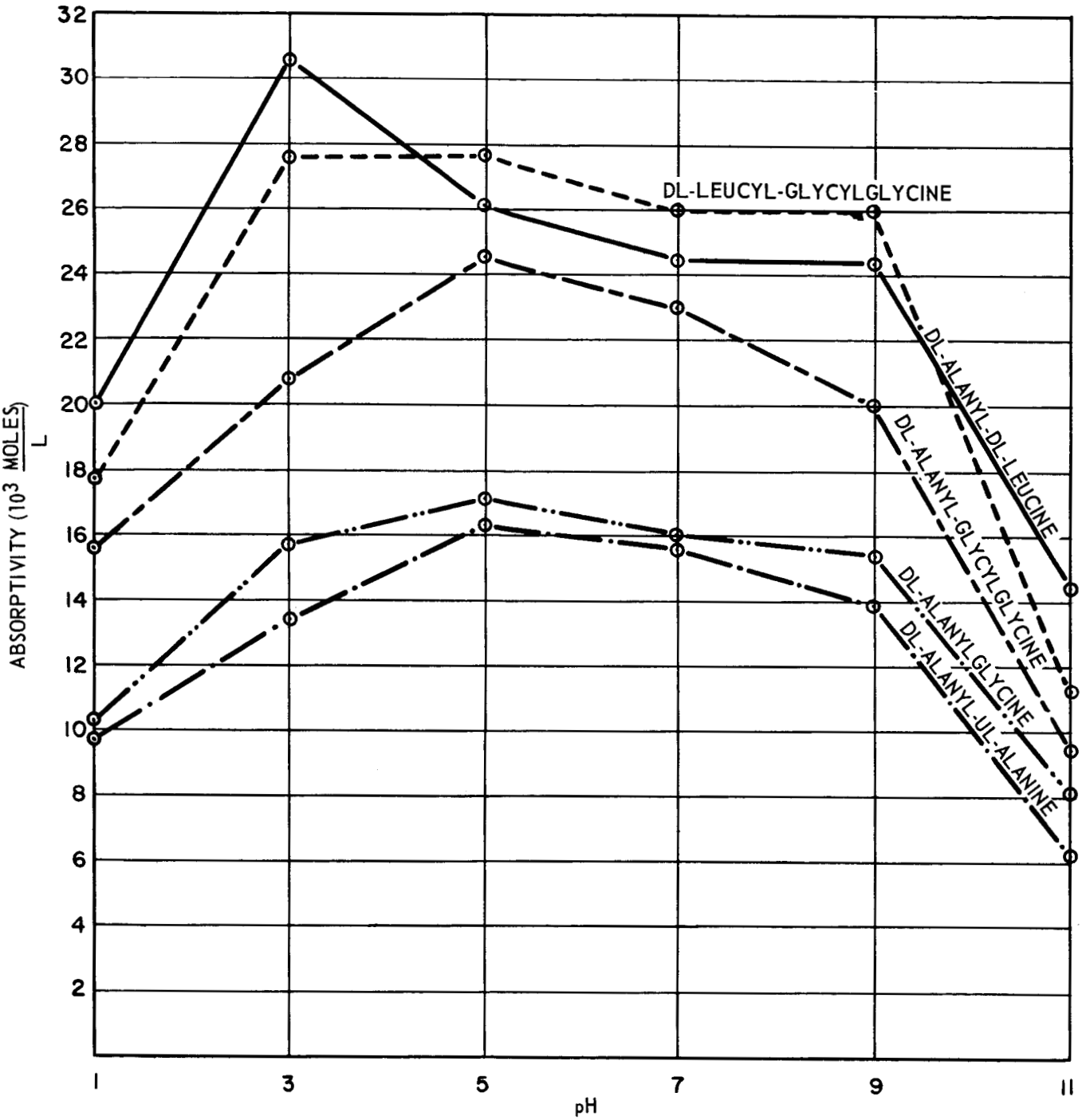

Figure 15. Effect of pH on Absorptivity by Peptides

a maximum at a pH of 3 to 7; for peptides containing leucine the maximum seems to occur at a lower pH.

Preliminary quantitative evaluations of the data were made to determine whether the absorption is truly characteristic of the peptide bond. It will be seen froom table I that, generally, the absorptivity is related to the number of peptide bonds. Thus, the polyglutamic acid containing 620 peptide bonds has an absorptivity 600 times greater than the alanylalanine and alanylglycine. Similarly, the polylysine preparation, having about 85 peptide bonds, has an absorptivity about 110 times as great as that of alanylalanine. In the case of leucyl-leucine, the dipeptide seems to behave like a tripeptide with regard to these spectrophotometric studies.

DISCUSSION

The appearance of the absorption maximum at 185-190 mμ in all of the peptides and proteins studied thus far suggests a relationship between the peptide bond and this absorption. However, the change in absorption with change in pH suggests that other mechanisms are also involved.

The change in absorption with pH may be associated with the helix-random coil transition as suggested by Rosenheck and Doty[3]. Another possible explanation for this effect is that the carboxyl ion contributes to the total absorption. This contribution is indicated by the low value absorption when the pH is reduced to 1. Under these conditions no ionized carboxyl group is expected. The precise mechanism is not of great

Table I

Material	ABSORPTIVITY (MOLAR) pH 1	pH 3	pH 5	pH 7	pH 9	pH 11
DL-alanyl-DL-alanine	1.36×10^3 *9.1×10^3	13.4×10^3	15.8×10^3 *16.4×10^3	15.7×10^3	17.7×10^3 *13.9×10^3	$.71 \times 10^3$ *6.1×10^3
DL-alanyl-glycine	1.26×10^3 *10.2×10^3	15.6×10^3	16.4×10^3 *17.2×10^3	16.2×10^3	12.5×10^3 *13.4×10^3	1.01×10^3 *7.9×10^3
DL-alanyl-DL-leucine	2.48×10^3 *17×10^3	30.4×10^3	26×10^3	24.4×10^3	20×10^3 *24.4×10^3	1.21×10^3 *14.4×10^3
DL-leucyl-glycyl-glycine	2.43×10^3 *17.7×10^3	27.5×10^3	27.5×10^3	26×10^3	20.3×10^3 *26×10^3	1.31×10^3 *11.1×10^3
DL-alanyl-glycyl-glycine	1.48×10^3 *15.4×10^3	26.8×10^3	24.4×10^3	23×10^3	16.5×10^3 *20×10^3	1.22×10^3 *9.35×10^3
Poly-L-lysine	1.66×10^5 *15.5×10^5	18.4×10^5	22.4×10^5 *24.2×10^5	16.5×10^5	12.5×10^5 *19×10^5	$.77 \times 10^5$ 10.4×10^5
Poly-glutamic acid				10×10^6		
Poly glycine	**2.9×10^5					
Bovine albumin	1.0×10^6 *6.25×10^6	8.3×10^6	1.8×10^6	7.8×10^6	6.0×10^6 *6.9×10^6	$.66 \times 10^6$ *4.9×10^6

* Indicates peaks other than at 190 wavelength

** pH was less than pH 1

significance in considering this phenomenon for detection of extraterrestrial life, but it takes on importance if the absorption is diagnostic of the presence of peptide bond. It then becomes necessary to know if the observed absorption is due to the peptide bond to or some other group that is not characteristic of biological systems.

The role that leucine plays in affecting the absorption of far ultraviolet is not clear yet. It was noted that leucine-containing peptides have a maximum absorptivity at pH 3 while others reached their maximum at a higher pH. It was also noted that alanylleucine had an absorptivity resembling that of a tripeptide. At this time it is not known what significance these observations may have.

The evidence obtained from the relationship of absorptivity to the number of peptide bonds lends weight to the hypothesis that the peptide bond is largely responsible for the absorption of far ultraviolet light. The approximate quantitative relationships obtained are especially interesting in this regard. The extent of the absorption points to the possibility that it might make a very sensitive detector of proteins and protein fragments. This is strikingly illustrated by the relative absorption at 190 mμ and 280 mμ exhibited by bovine serum albumin.

Anticipating the ultimate application of this phenomenon, it is interesting to note that extracts of local soil showed strong absorption bonds. The significance of this preliminary experiment lies in the detection of material that absorbs in the peptide region. However, it is to be noted that the soil seemed quite fertile. This type of experiment may become more meaningful when applied to infertile soils and normal aerosols.

SUMMARY

Absorption maxima between 185 and 190 mμ were found in dipeptides, tripeptides, polylycine polyglycine, and polyglutamic acid, bovine serum albumin and extracts of soil samples. All the absorptivities were pH-dependent going through a maximum between pH3 and pH7.

Present work includes an extension of these studies and a study of contributions to these absorptions by nonpeptide groups.

Following these studies, the practical application of the results to the detection of extraterrestrial life will be evaluated in terms of quantitative sensitivity specificity and interferences. The extension of the sensitivity will necessitate studies on solvents that are transparent to far ultraviolet light.

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